

Evaluation of liver biochemical parameters following administration of turmeric, vitamin C and alcohol among albino rats

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ABSTRACT

Background: The liver is an important organ involved in maintenance of gastrointestinal homeostasis and general body functions. Hepatotoxicity is liver damage or injury caused by drugs exposure and is grouped into hepatocellular, cholestatic and mixed, that results in an increase in alanine aminotransferase and alkaline phosphatase above the normal values.

Methodology: It was a posttest only true experimental design in which rats were grouped into five groups that included group I which had rats receiving rat pellets and water only, group II receiving alcohol, group III receiving alcohol and turmeric, group IV receiving alcohol and vitamin C and lastly group V receiving alcohol, turmeric and vitamin C. They were then subjected to alcohol, turmeric and vitamin C at calculated doses. Later on, blood sample was collected via cardiac puncture, centrifuged and liver biochemical analysis was done.

Results: A significant ($p < 0.0001$) increase was recorded in AST, ALT, GGT and albumin serum levels in alcohol treated group (group II) relative to negative control group (group I). Contrary, the serum levels of ALT and GGT significantly ($p < 0.0001$) decreased after treatments with individual turmeric and vitamin C and also in combined treatment with turmeric plus vitamin C. Combined treatment with vitamin C plus turmeric had a markedly significant effect (serum levels of AST, ALT, GGT and albumin) relative to their individual treatments. However, there was no significant difference in serum levels of AST and albumin in individual treatments with turmeric and vitamin.

Conclusion: It can therefore be concluded that Turmeric and vitamin C have a downward liver biochemical marker change on alcohol induced liver toxicity among albino rats.

Keywords: Alanine amino transferase; Aspartate amino transferase; Alkaline phosphatase gamma-glutamyl transferase; Hepatotoxicity and liver.

1. Introduction

Liver damage due to alcohol intoxication results in significant alterations in biomarkers of liver function. Serum aminotransferases, alanine and aspartate aminotransferases are the biomarkers commonly used to determine hepatotoxicity (Cho *et al.*, 2017). Liver injury biomarkers measure either an alteration in normal liver function, changes in tissue or cell integrity, detected by alanine amino transferase, aspartate amino transferase, alkaline phosphatase and gamma-glutamyl transferase (Andrade *et al.*, 2019). Alkaline phosphatase enzyme is found in liver and any increase in its level is caused by bile liver damage, flow obstruction or certain cancers. An increase in serum ALT is more specific for hepatocellular injury compared to increase in aspartate aminotransferase (AST) which also points at abnormalities in heart, muscle or kidney.

Ethyl glucuronide and ethyl sulfate in serum or urine, phosphatidyl-ethanol in blood and ethyl glucuronide and fatty acid ethyl esters in hair are several markers that have been proposed to extend the interval and sensitivity of detection. In ALD individuals, high levels of lipopolysaccharides in the liver affect the immune, parenchymal and non-immune cells which in turn release inflammatory cytokines and recruit neutrophils and other inflammatory cells. The mechanism by which alcohol contributes to activation of Kupffer cells and inflammatory cascade is characteristic for alcohol-induced DILI (Neuman, 2019).

Biochemical parameters of the liver are necessary to determine the level of hepatoprotective effect achieved by turmeric and vitamin C. Hepatotoxicity effect due to alcohol can also be determined by measuring the parameters.

The current study intends to base on the biochemical parameters to evaluate the effects of turmeric and vitamin C on the liver histo-architecture by comparing the biochemical parameters between the experimental group and control.

2. Materials and Methods

2.1. Experimental animals

In this study, albino rats of species *Rattus Norvegicus* were used due to their close biological and functional relation to human beings. The study design was a posttest only true experimental design in which an intervention was made and a comparison implemented. To assess the levels of liver biochemical parameters following administration of turmeric, vitamin C and alcohol, five (5) groups of albino rats were studied. The five groups included group I which had rats receiving rat pellets and water only, group II receiving alcohol, group III receiving alcohol and turmeric, group IV receiving alcohol and vitamin C and lastly group V receiving alcohol, turmeric and vitamin C.

2.2. Dose determination and administration

Dosage of turmeric was 40mg/ml (0.187mg/kg/day) adopted from previous study (Karamalakova *et al.*, 2019). Dosage of vitamin C was at 200mgs (0.3mg/kg) (Tawfik & Al Badr, 2012). Dosage of alcohol (ethanol) was at 3g/kg b.w (Boby *et al.*, 2021). Drugs were administered by use of gastric gavage needle i.e., the rats were wrapped with tablecloth and carefully held from the neck region by researcher. The research assistant then turned the mouth of the rat to face forward. Gently, the gavage needle was inserted into the mouth, drug delivered and then removed.

2.3. Harvesting and preparation of blood samples

Blood samples for biochemical parameters were harvested through a cardiac puncture after the animals were anaesthetized using carbon iv oxide. Blood was put in sterilized blood sample vacutainers and centrifuged. The analysis was done using AST/ALT/ALP ELISA KITS manufactured by Shenzhen Mindray Bio-Medical Electronics Company Limited in China. After preparing the reagents, samples and standards were added to the reaction at 37 °C for a period of 1hr and thirty minutes. The plate was washed, and the Biotinylated antibody working solution added, at 37 °C for 60 minutes. Then washed thrice and the Enzyme working solution added, at 37 °C for 30 minutes. Thereafter, washed five times, and then the Color Reagent solution was added, at 37 °C for 30 minutes. The Color Reagent C was added. Microplate reader was used to measure Optical Density values within 10 minutes. Then, the factor content of specimens tested was calculated.

2.4. Interpretation of Liver Assays Analysis – AST, ALT and ALP Results

Known concentrations of Rat ALT Standard and its accompanying reading OD was plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Rat ALT in sample was calculated by plotting the sample's O.D. on the Y-axis. The original concentration was found by multiplying the dilution factor. The normal ranges are AST 0.05 ng/ml-3.2 ng/ml, ALT 30 pg/ml -1500 pg/ml, ALP, 0.2 ng/ml - 15 ng/ml while

sensitivity results are AST 0.01 ng/ml, ALT 8 pg/ml and ALP 0.05 ng/ml therefore, the results obtained were compared to this normal ranges.

2.5. Ethical approval

Approval to carry out this study was sought from Eastern Africa University of Baraton (UEAB/ISERC/04/01/2023) and clearance by NACOSTI (NACOSTI/P/23/26476). All procedure pertaining this study were carried out based on guidelines and protocols for use and care of animals in biomedical research 2016.

3. Results

3.1. Effects of turmeric and vitamin C on alcohol induced changes in serum liver biochemical parameters (AST, ALT, GGT and albumin)

A significant ($p < 0.0001$) increase was recorded in AST, ALT, GGT and albumin serum levels in alcohol treated group (group II) relative to negative control group (group I). Contrary, the serum levels of ALT and GGT significantly ($p < 0.0001$) decreased after treatments with individual turmeric and vitamin C and also in combined treatment with turmeric plus vitamin C. Combined treatment with vitamin C plus turmeric had a markedly significant effect (serum levels of AST, ALT, GGT and albumin) relative to their individual treatments. However, there was no significant difference in serum levels of AST and albumin in individual treatments with turmeric and vitamin C (Table 1).

Table 1. Liver biochemical parameters (AST, ALT, GGT and albumin)

Liver Biochemical Parameters (u/l)	Group I (food +water)	Group II (Alcohol 3g/kgbw)	Group III (Alcohol+ Turmeric (0.187mg/kg/day)	Group IV (Alcohol+ vitamin C (0.3mg/kg/day)	Group V	df	F	sig
					Alcohol 3g/kgbw+ turmeric 0.187mg/kg/day+ vitamin C 0.3mg/kg/day)			
AST	29.41±.77	60.38±3.07#	42.7±1.77	39.96±2.72	27.68 ±.98**	4	19.827	.0001
ALT	64.18±4.16	103.0±4.97#	84.02±1.67*	88.80±2.53*	75.26±1.48**	4	52.545	.0001
GGT	2.54±.34	14.5±1.8#	4.2±.24	4.18±.50	3.42±.41**	4	31.66	.0001
ALBUMIN	4.4±.34	9.1±.91#	7.36±.20*	7.48±.15*	5.64±.24**	4	15.627	.0001

Key: Values are expressed as the means ± SEM; $n = 5$. # $P < 0.0001$ vs Normal control; * $P < 0.0001$ vs alcohol; ** $P < 0.0001$ vs alcohol. AST=aspartate transferase, ALT=alanine transaminase, GGT=gamma glutamate.

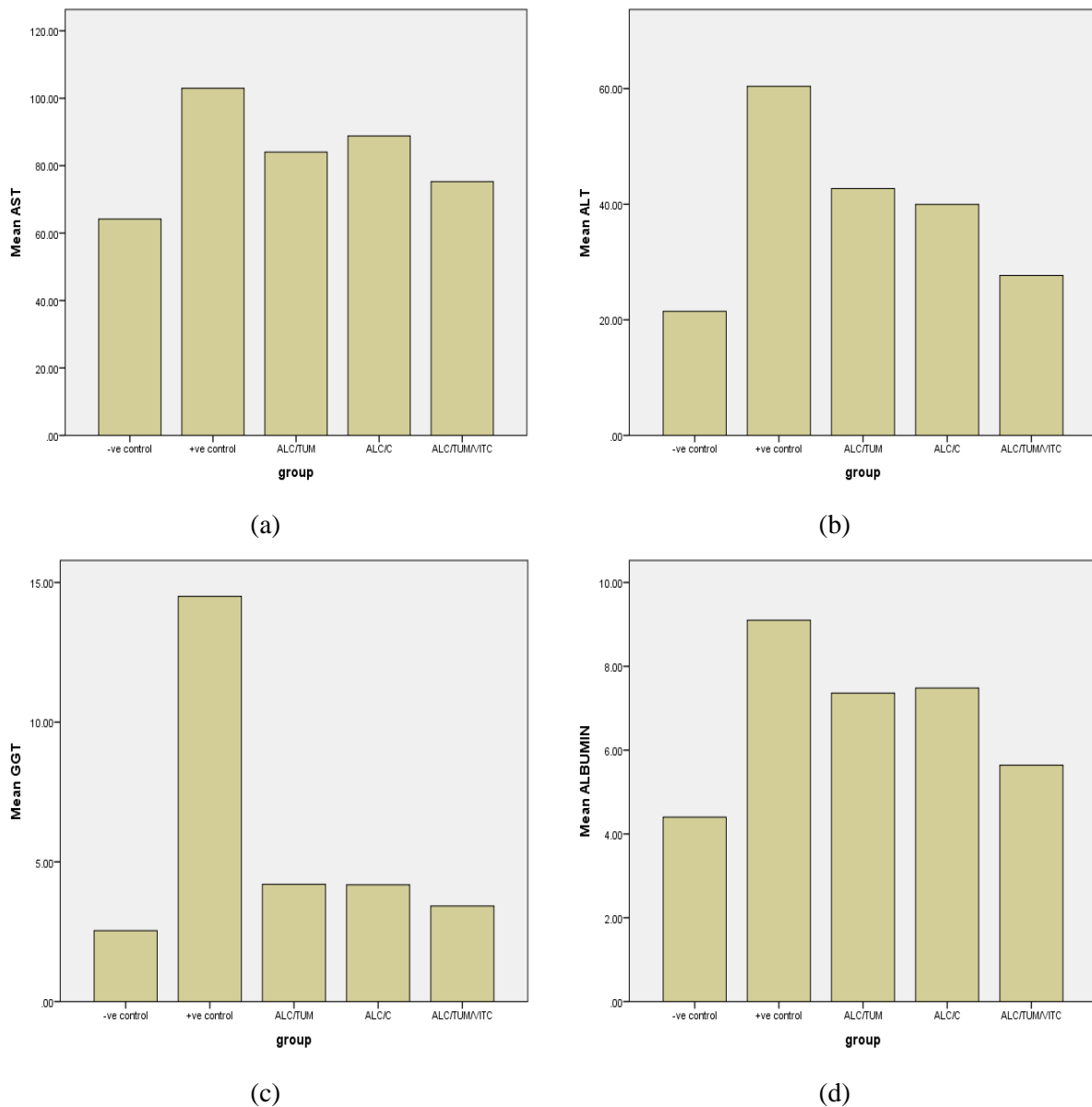


Figure 1. Graphical representation of liver biochemical parameters (AST, ALT, GGT and albumin)

4. Discussions

A significant increase was recorded in AST, ALT, GGT and albumin serum levels in alcohol treated group relative to negative control group. This significant increase in levels of AST, ALT, GGT and albumin are indicators of hepatotoxicity. Alcohol is known to cause these changes in biomarkers as it is normally metabolized in the liver.

The increase in these liver biochemical markers might have been due to increased cell injury and damage, changes in cellular integrity and obstructed flow of element as seen in (Andrade *et al.*, 2019; Cho *et al.*, 2017). These marked changes may also be attributed to alcohol effect on liver which might cause an inflammatory reaction thus release of inflammatory cytokines and other markers. This could potentially contribute to changes in activation systems of Kupfer cells thus forming an inflammatory cascade (Neuman, 2019).

Contrary, the serum levels of ALT and GGT significantly decreased after treatments with individual turmeric and vitamin C and also in combined treatment with turmeric plus vitamin C. These observations are similar to those of (García-Niño & Pedraza-Chaverrí, 2014; LS & HA, 2006). Where they noted that turmeric has antioxidant and anti-inflammatory benefits and thus might have protected liver damage and cellular changes from taking place. This would simply imply that the biomarkers will then drop as no cellular changes and Kupfer cell changes will take place. Combined treatment with vitamin C plus turmeric had a markedly significant effect (serum levels of AST, ALT, GGT and albumin) relative to their individual treatments.

However, there was no significant difference in serum levels of AST and albumin in individual treatments with turmeric and vitamin C (Table 1). Combined treatment of turmeric and vitamin C might yield better results when used synergistically or together. This is because the two substances are known to have anti-inflammatory and anti-oxidant benefits and could work together to suppress hepatotoxicity of the liver cells. It might also be postulated that the gross histomorphological changes witnessed due to their synergistic protection could potentially reduce the liver biochemical marker levels.

5. Conclusion

Based on this study, it can therefore be concluded that Turmeric and vitamin C have a downward liver biochemical marker change on alcohol induced liver toxicity among albino rats. This implies that they can be adopted for management in patients with alcohol hepatitis since they have proofed to physiologically lower the levels of biochemical parameters.

Declarations

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This research did not benefit from grant, any non-profit, public or commercial funding agency.

Competing Interests Statement

The authors have declared that no competing financial, professional or personal interests exist.

Consent for publication

All authors contributed to the manuscript and consented to the publication of this research work.

Ethical approval

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